Geographic variation in volatile leaf oils (terpenes) in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers)

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ABSTRACT

The composition of the steam volatile essential oils in leaves from 23 populations of *Helianthus annuus*, ranging from eastern Oklahoma to coastal southern California, were analyzed by GCMS. The oil compositions of populations from the southern high plains (KS, OK, TX) were uniform and dominated by α-pinene (50.5%), bornyl acetate (12.2), camphene (8.7), β-pinene (5.1), limonene (4.8) and sabinene (3.5). The oil composition from the quite distant San Diego was similar to the Plains populations: α-pinene (47.5%), sabinene (12.5), limonene (5.6), β-pinene (4.6), bornyl acetate (3.1) and camphene (2.7). Populations divergent from the Plains composition were Preston, ID: α-pinene (31.4%), bornyl acetate (21.0), limonene (7.2), camphene (6.7), and β-pinene (5.8); Redmond, OR: α-pinene (28.3%), bornyl acetate (16.8), germacrene D (8.2), limonene (7.2) and camphene (7.2); Eagle Nest, NM: α-pinene (27.3%), bornyl acetate (16.3), germacrene D (15.6), and β-pinene(5.3); and Camp Verde, AZ: germacrene D (19.1%), α-pinene (17.4), limonene (10.0) and β-phellandrene (6.7%). A population at Woodward, OK appeared to be an escaped commercial cultivar. Its oil was quite different and dominated by: β-pinene(27.3%) and germacrene D (25.4). Published on-line www.phytologia.org *Phytologia 99(2): 130-138 (May 9, 2017)*. ISSN 030319430.

KEY WORDS: *Helianthus annuus,* Sunflower, geographic variation in volatile essential oil yields, monoterpenes, sesquiterpenes, di-terpenes.

Annual sunflower (*Helianthus annuus* L.) is an important crop ranked as the second largest hybrid crop (only behind maize (*Zea mays* L.) with a global crop value of $20 billion (Seiler, Qi and Marek, 2017).

In spite of the enormous amount of research on sesquiterpene lactones in *Helianthus annuus* (a search of Google Scholar revealed 1,350 papers), we have found only two papers on the composition of volatile leaf essential oil. Ceccarini et al. (2004) reported on the composition of the steam volatile leaf and seed head essential oils of two *H. annuus* cultivars growing in Italy: 'Carlos' and 'Florom 350'. They reported the leaf oils of the two cultivars to be very similar with the major components being: α-pinene (28.2, 28.9%), sabinene (23.5, 23.2), limonene (11.1, 12.3), (iso) bornyl acetate (8.0, 7.9) and germacrene D (8.2, 8.8). From our survey (below) it seems likely that Ceccarini et al. found bornyl acetate rather than iso-bornyl acetate as they elute at about the same retention times and their MS differ by only mass 80 > 82 in bornyl acetate and 80 < 82 in iso-bornyl acetate. All our samples had mass 80 > 82, indicating the occurrence of bornyl acetate.

The second paper on essential oils of *H. annuus* (Ogunwande et al. 2010), examined the cultivated sunflower in Nigeria and reported the essential oil was dominated by α-pinene (16.0%), sabinene (9.4), germacrene D (14.4), and 14-hydroxy-α-muurolene (9.0).

Spring and Schilling (1989) published a thorough chemosystematic investigation of annual species of *H. annuus*, but their work was based on sesquiterpene lactones. There does not appear to be any information on geographic variation in the leaf essential (terpenes) oil of *H. annuus*. The purpose of this report is to present detailed analyses on the composition of steam volatile leaf terpenoids of *H. annuus* and on geographic variation in these oils. This is continuation of our research on sunflowers (Adams and TeBeest 2016, Adams et al. 2016, Adams et al. 2017).

MATERIALS AND METHODS

Population locations - see Adams et al. 2017.

The lowest growing, non-yellowed, 10 mature leaves were collected at stage R 5.1-5.3 (see Adams et al. 2017 for photos of sunflower growth stages) when the first flower head opened with mature rays. The leaves were air dried in paper bags at room temperature for transporting from the field, then kept frozen until analyzed. No doubt this resulted in some losses of the most volatile components (cf. α-pinene, etc.), but under the circumstances, this could not be avoided.

Isolation of Oils - Ten (10) leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS

Compositions of the steam volatile essential oils from leaves in several populations of *Helianthus annuus,* ranging from eastern Oklahoma to coastal southern California are given in Table 1. The compositions of populations from the southern high plains (Plains, Table 1, =KS, OK, TX) were uniform and dominated by α-pinene (50.5%), bornyl acetate (12.2), camphene (8.7), β-pinene (5.1), limonene (4.8) and sabinene (3.5). The quite distant San Diego population (SanD, table 1) had a composition similar to Plains populations: α-pinene (47.5%), sabinene (12.5), limonene (5.6), β-pinene (4.6), bornyl acetate (3.1) and camphene (2.7). Divergent populations from the Plains composition were Preston, ID (Pres ID, Table 1): α-pinene (31.4%), bornyl acetate (21.0), limonene (7.2), camphene (6.7) and β-pinene(5.8); Redmond, OR (Red OR, Table 1): α-pinene (28.3%), bornyl acetate (16.8), germacrene D (8.2), limonene (7.2) and camphene (7.2); Eagle Nest, NM (Eag NM, Table 1): α-pinene (27.3%), bornyl acetate (16.3), germacrene D (15.6), and β-pinene(5.3); and Camp Verde, AZ (Ariz, Table 1): germacrene D (19.1%), α-pinene (17.4), limonene (10.0) and β-phellandrene (6.7%). A population at Woodward, OK appeared to be an escaped commercial cultivar. The oil was quite different (WO, Woodw, Table 1) and was dominated by: β-pinene (27.3%) and germacrene D (25.4).

Fifteen (15) of the major terpenoids (boldface, Table 1) were utilized for computing similarities among populations (Gower, 1971). Factoring the similarity matrix resulted in five eigenroots before they began to asymptote. The five eigenroots accounted for 24.3, 15.4, 10.3, 7.5 and 7.1% of the variation among the populations. Principle Coordinates (PCO) of the similarity matrix revealed the major group as the Plains populations (Fig. 1) with the Woodward, OK (WO) population ordinated on axis 2. The plants in the Woodward population had very large, glabrous (no pubescence) leaves and larger seed heads than other populations. It appears that these are likely hybrids with commercial sunflowers. As noted above (and in Table 1), their essential oil is quite different from other *H. annuus* sampled. That is clearly seen in the PCO ordination (Fig. 1). The third axis serves to separate the more western populations from the Plains populations (Fig. 1).

Removing the Woodward, OK (WO) population and reanalyzing the terpenes resulted in five eigenroots that accounted for 27.6, 11.3, 8.6, 8.1, and 6.4% of the variance among the populations. Ordination (Fig. 2) shows a pattern similar to the previous pattern (Fig. 1), with the divergence western populations better resolved from the Plains cluster. Interestingly the San Diego populations (small leaves, SS, and large leaves SL) were near or within the Plains cluster (Fig. 2).

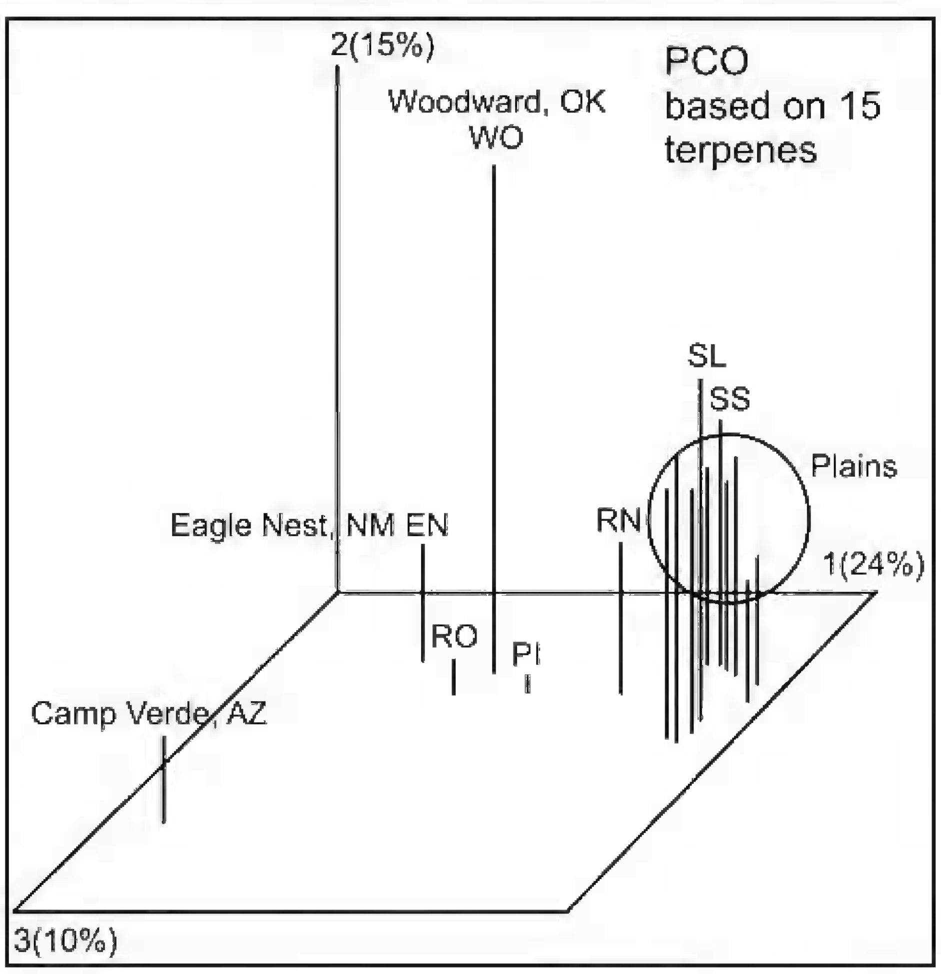

Figure 1. PCO based on 15 terpenes with Woodward (WO) included.

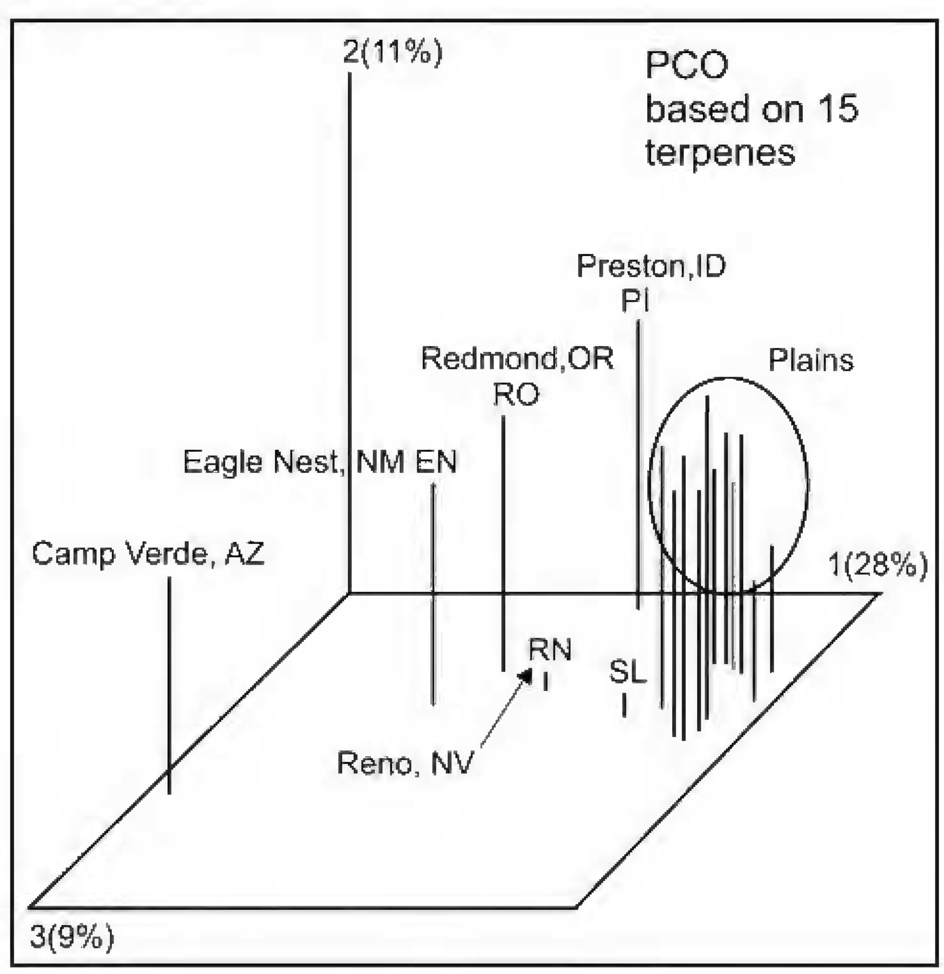

Figure 2. PCO based on 15 terpenes without the Woodward population.

Contouring the clustering of the populations clearly shows the geographic variation pattern (Fig. 3. The populations with the most similar volatile leaf oil compositions are those in the Plains group: KS, OK and ST (Sonora, TX, but grown from seed in the Texas Panhandle at Oslo, TX). Also closely joining the cluster are Texas Panhandle, south Plains and Central Texas (MC) populations (Fig. 3). The clustering of the disjunct sunflowers from San Diego is an anomaly. This kind of disjunct clustering is also seen for the Redmond, OR (RO) and Brigham City, UT (BU) populations, to some extent. Just as depicted in the PCO (Figs. 1, 2), Camp Verde, AZ (AZ, Fig. 3) is the last population to enter the cluster at a similarity of 0.68 (Fig. 3).

The San Diego populations might owe their origin to historical dispersal by settlers moving westward from Kansas on the California trail, who accidentally (or on purpose) carried seed from the Prairies to California. Or it may be that Native Americans dispersed the Plains seed into the western United States.

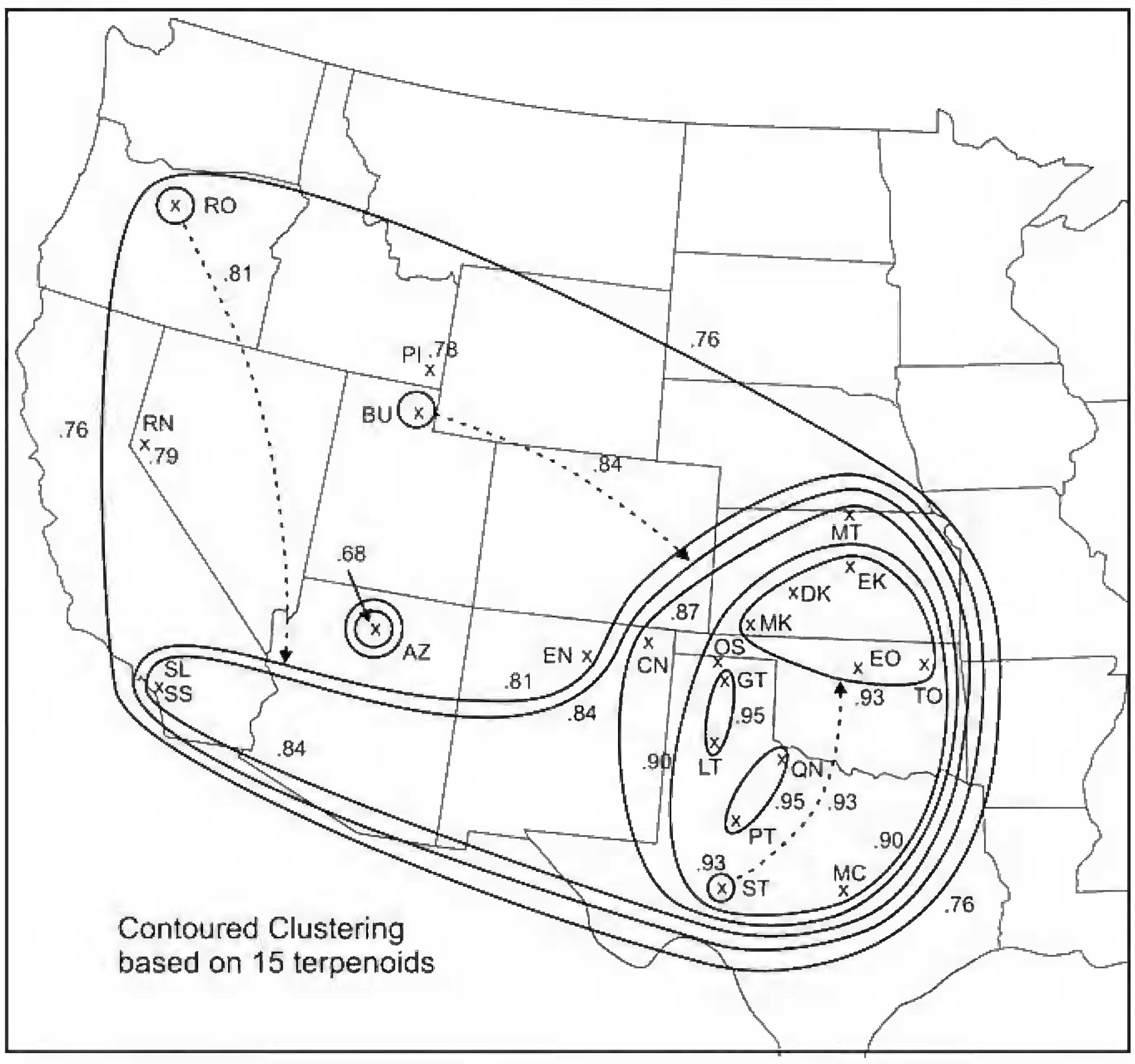

Figure 3. Contoured clustering of populations utilizing 15 major terpenes.

The yields of essential oils were computed as % yield and as g oil/ g of 10 air dried steam distilled leaves. The Montrose, KS (MT) population had the highest % yield of total essential oil (3.03%, Table 2, Fig. 4), followed by Capulin, NM (CM, 1.85%), Gruver, TX (GT, 1.47%), and Lake Tanglewood, TX (LT, 1.21%). The range of variation in % oil was quite extensive, from 3.03% (Montrose, KS) to 0.22% (Camp Verde, AZ).

The sunflowers growing in a prairie grassland at Oslo, TX (OS) population (which is only 15 miles from Gruver, TX) interestingly had a much lower % oil yield (0.80%) than plants growing on a roadside Gruver (1.21%). This may be due to an environmental influence on % oil yields rather than genetic differences. The sunflowers at Gruver were extensively attacked and eaten by grasshoppers and other insects. This may have triggered a defense mechanism to increase terpene (defense chemicals) synthesis. The large yield in the Montrose population was unexpected, but it should be noted that seeds from Montrose, KS were grown at Oslo, TX, from which leaves were collected. So, one can not be sure that the data represents what the MT would have produced *in situ*.

A different pattern of geographic variation exists in total oil yields (g oil/ g DW of 10 distilled leaves). Again, the largest yield was in the Montrose population (MT, 0.310 g, Table 2, Fig. 5), followed by Lake Tanglewood (LT, 0.225g), Gruver (GT, 0.223g), and Enid (EO, 0.206g). The Enid population was not very high in % oil yield (0.8%), but the plants were very large, resulting in a high g oil /10 leaves (0.206g).

The variation among populations in g oil/ g 10 leaves is greater than variation in % oil yields (Fig. 5, vs. Fig. 4). This is reasonable, as g oil/ g 10 leaves is greatly affected by the biomass of the plants, which is subject to microhabitat edaphic factors. Montrose, Lake Tanglewood, and Gruver were high in both % oil yields and g oil/ g DW 10 leaves (Table 2, Figs. 4, 5).

The correlation between % oil yields and % HC yields was low 0.339, explaining only 11.5% of the variation among populations. The correlation between g/g 10 leaves yields of essential oil and HC g/g 10 lvs was 0.675. This correlation accounts for 45.6% of the variation among populations. It may be that that the yields on a g/ g basis are highly influenced by environmental factors that are local to the population level, whereas, % yields are less influenced by environmental factors. I (RPA) have found that the yield of HC from greenhouse grown plants is only about 40% as much as from plants grown outside in the natural environment.

Clearly, more research is needed to determine the environmental versus genetic factors in the production of essential oils in *H. annuus*.

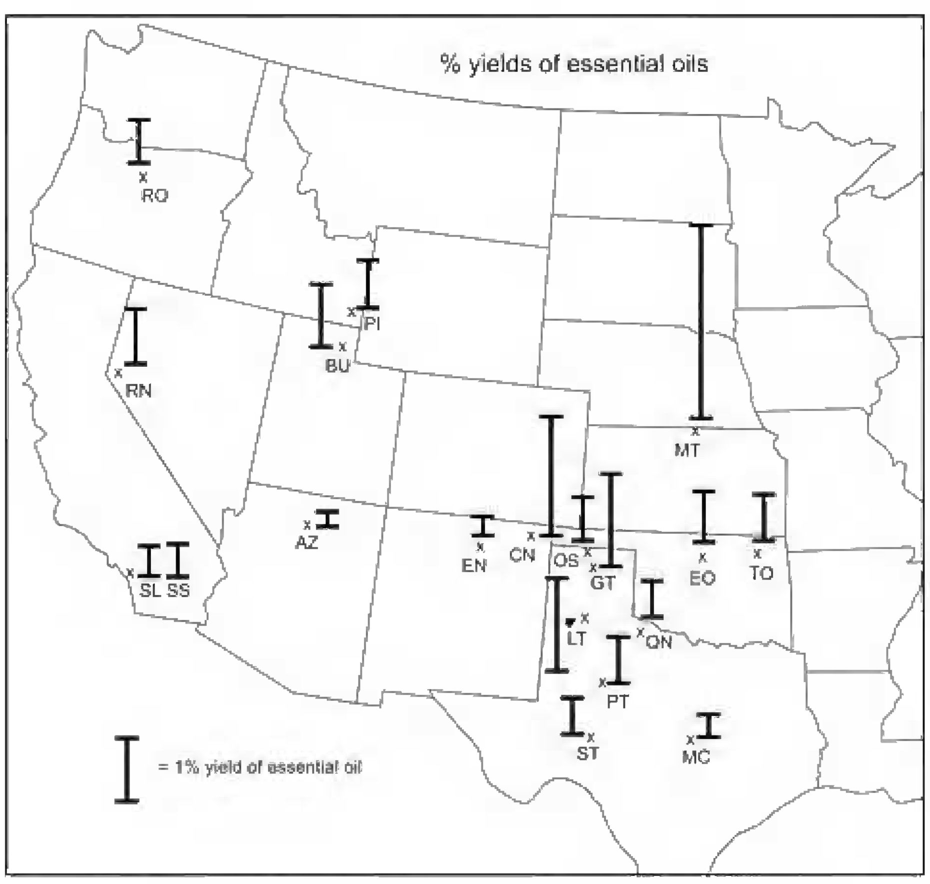

Figure 4. Plot of % oil yields. Note scale in lower left.

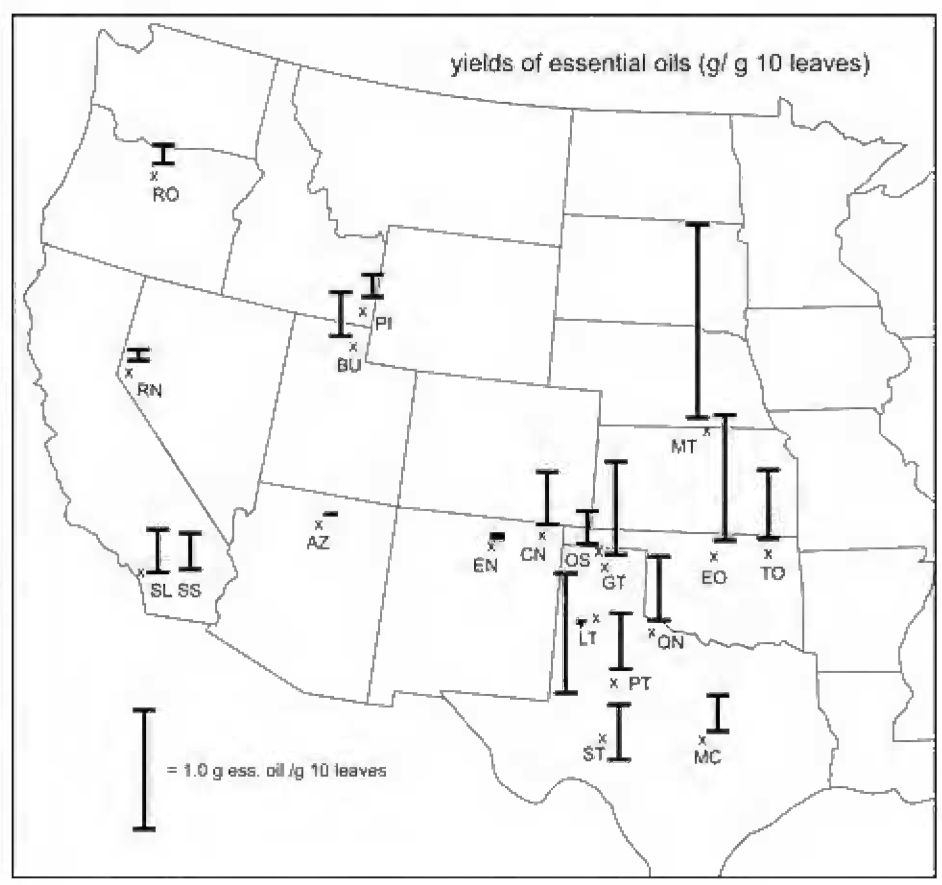

Figure 5. Plot of g oil/ g wt. of 10 dried leaves. Scale is in lower left.

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Table 1. Leaf essential oil compositions for *Helianthus annuus* populations. The 15 compounds in bold show large differences between samples and were used in PCO analysis. Plains = general pattern found in the Texas Panhandle, Kansas and western Oklahoma. Woodw = population of cultivated escaped sunflowers at Woodward, OK, Ariz = Camp Verde, AZ, SanD = San Diego.

KI	compound	Plains	SanD	Reno	Pres ID	Red OR	Eag NM	Ariz	Woodw
921	tricyclene	0.2	0.1	0.2	0.2	0.2	t	t	t
924	α-thujene	0.4	0.1	t	0.2	0.1	t	t	t
932	**α-pinene**	**50.2**	**47.5**	**37.3**	**31.4**	**28.3**	**27.3**	**17.4**	**6.8**
946	**camphene**	**8.7**	**2.7**	**4.5**	**6.7**	**7.2**	**3.5**	**0.9**	**2.2**
969	**sabinene**	**3.5**	**12.0**	**2.3**	**3.0**	**2.3**	**1.7**	**4.8**	**6.3**
974	**β-pinene**	**5.1**	**4.6**	**4.9**	**5.8**	**4.6**	**5.3**	**3.2**	**27.3**
988	myrcene	0.3	0.7	0.4	0.7	0.5	0.8	0.8	0.3
1005	(2E,4E)-heptadienal	t	-	-	t	-	-	-	0.1
1014	α-terpinene	t	-	-	t	-	t	-	t
1020	p-cymene	t	0.1	t	0.2	t	t	t	t
1024	**limonene**	**4.8**	**5.6**	**4.5**	**7.2**	**7.2**	**2.6**	**10.0**	**0.9**
1025	**β-phellandrene**	**t**	**3.8**	**3.0**	**5.0**	**4.6**	**1.8**	**6.7**	**0.6**
1036	benzene acetaldehyde	-	0.1	0.3	0.1	0.1	t	0.2	-
1044	(E)-β-ocimene	-	t	t	t	t	t	t	0.3
1054	γ-terpinene	0.1	0.1	t	0.3	0.1	0.2	0.1	0.1
1065	cis-sabinene hydrate	t	t	-	0.1	t	t	t	0.1
1086	terpinolene	-	0.1	t	0.2	0.1	0.3	t	t
1098	trans-sabinene hydrate	-	-	-	0.2	-	t	0.1	t
1099	α-pinene oxide	0.3	0.2	0.4	-	-	-	-	-
1105	43,109,137,152	0.5	0.5	1.4	0.4	0.1	0.4	-	-
1123	α-camphenal	0.3	0.3	0.5	0.2	-	t	-	t
1135	trans-pinocarveol	0.1	0.3	0.8	0.1	t	0.2	0.1	0.6
1137	cis-verbenol	0.1	-	-	0.1	-	-	-	-
1140	**trans-verbenol**	**1.0**	**1.0**	**1.9**	**0.6**	**0.3**	**0.7**	**0.6**	**0.2**
1160	pinocarvone	0.2	0.2	0.3	0.2	t	t	t	0.2
1165	borneol	0.4	t	0.4	0.3	0.1	3.3	t	7.5
1174	terpinen-4-ol	0.1	-	-	0.2	-	0.2	t	0.4
1179	p-cymen-8-ol	t	-	-	-	-	-	t	t
1186	α-terpineol	t	-	-	-	-	-	-	0.1
1195	myrtenal	0.2	0.1	0.2	0.1	-	t	-	0.1
1195	myrtenol	t	0.2	0.5	-	-	-	t	0.1
1204	verbenone	0.2	-	0.2	0.1	-	t	-	-
1212	55,83,135,154	-	0.1	-	-	-	-	-	0.3
1215	trans-carveol	-	t	0.2	-	-	-	-	-
1287	**bornyl acetate**	**12.2**	**3.1**	**9.7**	**21.0**	**16.8**	**16.3**	**4.2**	**5.5**
1298	trans-pinocarvyl acetate	0.2	0.2	0.5	0.3	0.3	0.3	t	-
1324	myrtenyl acetate	-	t	0.1	-	-	-	-	-
1356	eugenol	-	-	-	-	-	-	-	0.2
1374	α-copaene	-	-	-	-	-	t	t	0.1
1387	β-bourbonene	0.1	t	1.5	t	0.1	0.2	0.3	0.2
1387	β-cubebene	t	-	-	t	-	0.2	0.4	t
1389	β-elemene	t	-	-	-	-	t	0.1	0.2
1392	(Z)-jasmone	-	-	-	-	-	-	-	0.2
1417	**(E)-caryophyllene**	**0.2**	**0.2**	**0.3**	**0.1**	**0.5**	**1.0**	**1.0**	**2.7**
1430	β-copaene	-	t	0.2	-	-	-	t	0.1
1432	α-trans-bergamotene	0.1	t	0.2	0.1	-	t	t	-
1439	aromadendrene	-	-	0.2	-	-	-	-	-
1452	geranyl acetone	0.1	0.2	0.1	-	0.1	t	0.2	-
1452	α-humulene	0.1	t	t	0.3	0.3	0.6	0.5	0.6
1480	**germacrene D**	**2.8**	**2.6**	**0.5**	**1.9**	**8.2**	**15.6**	**19.1**	**25.4**
1489	β-selinene	0.1	-	-	0.2	-	0.2	-	-
1493	epi-cubebol	-	0.2	0.1	t	0.2	t	-	-
1500	bicyclogermacrene	-	-	-	-	-	-	-	0.9

KI	compound	Plains	SanD	Reno	Pres ID	Red OR	Eag NM	Ariz	Woodw
1513	γ-cadinene	-	-	-	t	-	t	0.2	t
1522	δ-cadinene	-	-	-	t	-	0.3	0.3	0.1
1559	**germacrene B**	**-**	**-**	**-**	**1.1**	**-**	**0.4**	**1.1**	**-**
1561	1-nor-bourbonene	-	-	-	-	-	-		0.3
1574	germacrene D-4-ol	0.2	-	-	0.1	-	1.5	1.1	-
1577	spathulenol	0.2	t	0.4	t	-	t		-
1582	caryophyllene oxide	0.2	0.1	0.4	0.2	-	0.3	0.2	1.0
1594	salvial-4(14)-en-1-one	-	0.1	0.2	-	-	-	-	-
1602	41,95,161,222	-	-	-	t	-	-	0.7	-
1608	humulene epoxide II	0.2	-	-	-	-	0.5	-	1.2
1618	junenol	t	-	-	-	-	-	-	-
1638	epi-α-cadinol	t	-	0.6	0.3	0.2	0.9	0.1	-
1649	β-eudesmol	0.3	-	0.4	0.5	0.5	0.6	-	-
1665	**intermediol**	**0.4**	**0.6**	**0.6**	t	**0.3**	**0.4**	**0.2**	**1.6**
1685	**germacra-4(15),5,10(14)-trien-1-al**	**0.3**	**0.5**	**2.3**	**0.3**	**0.6**	**0.8**	**1.8**	**0.2**
1780	ledene oxide II*	-	0.6	1.4	t	0.3	0.6	0.9	-
1840	43,68,123,278	0.5	0.4	0.8	0.4	0.8	0.4	1.1	-
1840	hexahydrofarnesyl acetone	-	-	-	-	-	-	-	0.1
1931	beyerene	0.1	-	-	-	-	-	t	-
2009	13-epi-manoyl oxide	t	-	-	t	-	t	0.1	-
2114	phytol isomer	-	-	-	-	-	t	0.3	0.4
2148	41,91,105,135,286	0.3	-	0.5	0.5	0.4	0.4	1.4	-
2192	p-methoxybenzoic acid, 2-isopropoxyphenyl ester, isomer	-	-	-	-	-	-	-	0.4
2205	41,91,105,286	t	t	0.3	0.2	0.2	0.2	0.6	-
2216	atis-16-ene* <5β,8α,9β,10α,12α->	**0.4**	**0.3**	**0.9**	**0.5**	**0.7**	**0.6**	**1.3**	**-**
2220	43,123,272,290	0.2	0.2	0.5	0.3	0.4	0.3	0.6	-
2235	43,232,272,290	t	0.1	0.3	0.3	0.3	0.3	0.6	-
2241	**105,135,257,288**	**0.5**	**0.1**	**0.9**	**0.7**	**1.0**	**0.5**	**1.4**	**-**
2253	41,257,91,286	-	0.1	0.2	0.4	0.2	0.3	0.9	-
2263	91,187,243,286	0.3	0.3	0.9	0.7	0.6	1.0	3.1	-

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. *tentatively identified from NIST mass spectral database.

Table 2. Comparison of the yields volatile leaf oils and hydrocarbons (HC) for *H. annuus*, from natural populations. Correlation between % yields of essential oil and HC = 0.339; Correlation between g/g 10 leaves yields of essential oil and HC = 0.675.

popn id, sample ids	population sampled	volatile oil, % yield	HC, % yield	volatile oil g/10 lvs	HC yield g/10 lvs
PT P1 - P0	14935 Post, TX	0.81	4.36	0.095	0.481
QN Q1-Q0	14936 Quanah, TX	0.69	4.32	0.119	0.779
TO T1-T0	14942 Tulsa, OK	0.60	4.56	0.114	0.778
EO O1-OT	14943 Enid, OK	0.84	4.97	0.206	1.094
WO W1-W0	14944 Woodward, OK, (escaped cultivar)	0.25	2.62	0.047	0.644
ST S1-S0	14945 grown from seed, ex Sonora, TX, PI413168	0.70	5.19	0.089	0.744
OS O1-TO	14946 Oslo, TX,	0.80	5.95	0.082	0.508
LT: L1-L0	14947 Lake Tanglewood, TX	1.21	8.47	0.225	1.760
SS SA-SJ	14950 San Diego, CA, small leaves	0.55	4.59	0.035	0.335
SL SK-ST	14951 San Diego, CA, large leaves	0.47	4.68	0.056	0.54
GT1:G1-G0	14952 Gruver, TX	1.47	7.26	0.223	1.54
MC 1M-0M	14976 McLennan Co., TX	0.36	6.18	0.058	0.923
EN 1E-0E	14980 Eagle Nest, NM	0.31	2.62	0.013	0.143
CN 1C-0C	14981 Capulin, NM	1.85	5.29	0.098	0.232
MT MA-MC	14982 grown from seed ex Montrose, KS, PI 413033	3.03	4.91	0.310	0.518
AZ Z1-Z0	15021 Camp Verde, AZ	0.22	3.79	0.007	0.213
BU B1-B0	15022 Brigham City, UT	1.01	5.90	0.060	0.420
PI 1P-0P	15024 Preston, ID	0.80	6.30	0.028	0.338
RO R1-R0	15027 Redmond, OR	0.72	4.06	0.028	0.291
RN R1-R0	15029 Reno, NV	0.96	5.11	0.016	0.178